TO THE EUROPEAN PATENT OFFICE

Re: Opposition to European Patent 0 125 023 (Genentech, Inc.)

DECLARATION OF JEFFREY V. RAVETCH, M.D., Ph. D.

I, Jeffrey V. Ravetch, M.D., Ph.D. declare and state as follows:

1. I am a Professor at The Rockefeller University, Head of the Laboratory of Molecular Genetics and Immunology, and an Adjunct Professor in the Department of Microbiology and Immunology at Jefferson Medical College and Jefferson Cancer Institute. Prior to July 1996, I was an Attending Physician in the Division of Hematologic Oncology (Department of Medicine) at Memorial Hospital for Cancer and Allied Disease, and a Professor at Cornell University Medical College.

2. In 1973, I acquired a Bachelor's Degree of Science in molecular biophysics/biochemistry from Yale College. In 1978, I received a Ph.D. from the Laboratory of Genetics at The Rockefeller University and in 1979, a Medical Doctorate from Cornell University Medical College. My education, experience, patents, and publications are further set forth in the attached curriculum vitae (Exhibit "A").

3. Since 1978, I have closely followed the academic literature regarding antibody structure and synthesis. The authors of these works are predominantly molecular biologists with backgrounds in immunology, immunologists and biochemists. Since 1979, my research and scholarship have concentrated in molecular biology and immunology. Some of my scholarly endeavors pertain to antibody structure. My laboratory has characterized immunoglobulin genes and Fc receptors. This

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research has included molecular cloning, chimeric antibody constructions, and other recombinant antibody technology.

4. I am familiar with the disclosure and claims of the above-identified European patent ("the Cabilly patent"). I am also familiar with the opposition briefs filed by Boehringer Mannheim GmbH (Opponent III), Protein Design Labs, Inc. (Opponent IV), Celltech Limited (Opponent VI), including the submission dated February 20, 1997, filed by Opponent VI.

5. It is my understanding that certain Opponents question the sufficiency of the disclosure provided in the Cabilly patent for making chimeric antibodies.

5.1 Opponent III specifically challenged the sufficiency of disclosure provided in the examples since the Wagener *et al.* paper, cited on page 14, line 14 as describing the mouse hybridoma cell line used as a source of the anti-CEA antibody mRNA, was not publicly available at the priority date of the Cabilly patent.

5.2 Opponent IV pointed out that the restriction enzyme *Ava*III was not commercially available at the priority date of the Cabilly patent and would have been difficult to purify. In addition, according to Opponent IV the site-directed deletional mutagenesis step described in Example E.4 could not have been performed at the priority date of the Cabilly patent since the Adelman *et al.* paper referenced on page 21, lines 55-56 was published only at a later date.

5.2 In its submission dated February 20, 1997, Opponent VI suggests that the preparation of chimeric antibodies is not sufficiently described in example E.4, since (1) the sequence of the anti-CEA antibody was not available at the priority date of the above-identified patent; (2) the vectors proposed for expressing the cDNA sequences encoding the chimeric light and heavy chains, respectively contain the same origin of replication, which would lead to the ejection of one of these vectors due to plasmid incompatibility, should one introduce them into a single bacterial host cell; and (3) a "fully chimeric chain" could not have been prepared following the directions provided in E.4, since

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the mp7 vector described in the Messing *et al.* paper cited in the specification (Nucleic Acids Res. 9:309 (1981), cited on page 21, lines 54-55) does not contain an *Xba*I or *Pvu*II site in a cloning site.

6. 6.1 I have reviewed the specification of the Cabilly patent, including examples E.1.1 through E.1.10 and E.4. Based upon my review and my general knowledge of the state of the art as it existed in 1983, I disagree with the Opponents' position concerning the sufficiency of disclosure. It is my considered scientific opinion that a chimeric antibody could have been prepared following the directions provided in the Cabilly patent at the priority date of April 3, 1983.

6.2 Examples E.1.1 through E.1.10 describe the steps of the production of antibodies to the carcinoembryonic antigen (CEA) ("anti-CEA antibodies") by recombinant DNA technology. These steps include the isolation of messenger RNA (mRNA) for anti-CEA antibody light and heavy chains (E.1.1), the cloning of cDNAs encoding anti-CEA antibody light and heavy chains (E.1.2 - E.1.6), the construction of expression vectors for the direct expression of anti-CEA antibody light and heavy chains, respectively (E.1.7 and E.1.8), the recombinant production of anti-CEA antibody chains by expression in *E. coli* cells transformed with expression vectors encoding the respective chains (E.1.9), and the reconstitution of the antibody chains (E.1.10).

6.3 Example E.4 describes the preparation of a chimeric anti-CEA antibody which comprises a murine anti-CEA antibody variable region and a human antibody constant region.

7. 7.1 The first part of example E.4 describes the construction of an expression plasmid for a chimeric anti-CEA antibody heavy chain comprising the coding sequence for the variable part of the murine anti-CEA antibody γ1 (IgG-1) chain and human γ2 (IgG-2) constant region. The nucleotide sequence encoding the human γ2 chain was described by Ellison *et al.*, Proc. Natl. Acad. Sci. USA 79:1984 (1982) (Exhibit "B"). The complete nucleotide sequence encoding the murine anti-CEA antibody γ1 chain is shown in Figure 4. The mRNA encoding an anti-CEA antibody γ1 chain could have been readily isolated by a skilled molecular biologist at the priority date of the Cabilly patent,

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following the instructions provided in example E.1. The carcinoembryonic antigen (CEA) was a well known marker for human colon cancer at the time. Numerous papers, including Accolla *et al.*, Proc. Natl. Acad. Sci. USA, 77:563 (1980) (Exhibit "C"), Kupchik *et al.*, Cancer Res., 41:3306 (1981) (Exhibit "D"), and Hedin *et al.*, Mol. Immunol. 19:1641 (1982) (Exhibit "E"), described hybridoma cell lines producing anti-CEA monoclonal antibodies of high affinity. Accordingly, a researcher had a number of hybridoma anti-CEA antibody producing cell lines available at the priority date of the Cabilly patent from which mRNA encoding such antibody could have been isolated following routine techniques, as described in example E.1. The construction of an expression plasmid for a chimeric anti-CEA antibody heavy chain (pChimI) is described on page 21 of the patent, and is illustrated in Figure 11. Although the generation of the desired portion of the human γ2 gene involved the use of the restriction enzyme *Ava*III, which could have been purified by techniques known at the time, the same result could have been readily achieved by relying on other restriction sites present in the γ2 chain. Alternatively, for convenience, new unique restriction sites could have been created by site directed mutagenesis through the use of appropriate short synthetic oligonucleotide sequences. As the plasmid pChimI contains murine variable region and human constant region coding sequences, it will in fact express a chimeric antibody heavy chain when transformed into appropriate recombinant host cells, e.g. *E. coli*. The fact that, in the chimeric antibody chain expressed, the change from murine to human sequences does not take place at the exact variable to constant region junction does not take away from its chimeric nature. This is clearly expressed at page 21, lines 41-42 of the patent, where it is stated that "pChim1 will, in fact, express a chimeric heavy chain when transformed into E. coli, but one wherein the change from mouse to human does not take place at the variable to constant region junction." Indeed, there is no reason to believe that an antibody comprising such chimeric chain would not be fully functional.

7.2 Figure 12 shows the further modification of the plasmid pChimI to provide a plasmid (pChimII) containing the coding sequence of a second chimeric antibody heavy chain, in which the change from murine to human sequences takes place at the exact variable to constant region junction.

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The construction of this second chimeric antibody heavy chain proceeds through an intermediate plasmid (referred to as pIntermediate human/mouse hybrid in Figure 12), which contains a short extraneous sequence, consisting of a small portion of the variable region of the human γ2 heavy chain, and a small portion of the constant region of the murine anti-CEA antibody γ1 heavy chain. According to the instructions provided at pages 21-23 and in Figure 12, this extraneous sequence is removed by excising the *Xba*I to *Pvu*II fragment of the plasmid, cloning in an M13 phage vector, ***in vitro*** site directed deletion mutagenesis to provide the correct junction sequence, followed by cloning of the modified, correct *Xba*I to *Pvu*II fragment into the XAP plasmid to form pChimII. The complete nucleotide and amino acid sequences of the murine anti-CEAγ1 chain are shown in Figures 4 and 5. The nucleotide and deduced amino acid sequences of human antibody γ2 heavy chain constant region were described in Ellison *et al.*, *supra* (Exhibit "B"). Based upon this information, a person of ordinary skill in the art at April 8, 1983 would have well known that in the coding sequence present in the intermediate plasmid shown in Figure 12 any segment present in addition to the coding sequence of the human antibody heavy chain constant region and the murine anti-CEA antibody heavy chain variable region is extraneous, and needs to be excised to obtain an accurate variable region - constant region junction in the chimeric antibody heavy chain. A skilled person would also have known how to perform the excision. Site-directed deletion mutagenesis was a technique known in the art at the priority date of the Cabilly application. For example, Messing *et al.*, Nucleic Acids Res. 9: 309 (1981) (Exhibit "F"), cited at page 21, lines 54-55 of the Cabilly patent, teaches the use of M13 for site-directed mutagenesis in general. A specific version of this technique, deletion mutagenesis, was also well known in the art at the priority date - see, for example, Wallace *et al.*, Science 209:1396-1400 (1980) (Exhibit "G"). I note that the Cabilly patent does not call for the use of the phage vector M13mp7 described by Messing *et al.*, or of any other specific phage vector. It is clear to me that the instructions to clone the *Xba*I to *Pvu*II fragment into "M13 phage *as described* by Messing *et al.*" (page 21, line 54, emphasis added) mean just what they say, the cloning of the fragment into an M13-based expression vector, following the approach described by Messing *et al.* In 1983, this could have been achieved in at least three ways, or by the combination

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of any of these three approaches. First, an M13 vector having a cloning site including the *Xba*I and *Pvu*II restriction sites could have been constructed following the teaching of Messing *et al.* Second, the ends of the *Xba*I-*Pvu*II fragment could have been modified by the ligation of synthetic adapters, which would have enabled the insertion of this fragment into the cloning site of an existing M13 vector, such as M13mp7. Third, restriction sites present in an existing vector, such as M13mp7, could have been converted into the required *Xba*I and *Pvu*II restriction sites by site-directed *in vitro* mutagenesis. As mentioned before, the latter technique was well known in the art at the priority date of the Cabilly patent, and is also illustrated in Figure 3 of Messing *et al.* It is, therefore, my considered opinion that a person of ordinary skill in the art at the priority date of the Cabilly application had the ability to prepare a correct variable domain - constant domain junction in the coding sequence of the disclosed chimeric immunoglobulin, without undue experimentation.

8. According to example E.4 of the Cabilly application, the expression plasmid for a chimeric antibody light chain is prepared by analogy to the preparation of the chimeric heavy chain expression plasmid, and the two plasmids (harboring coding sequences for the chimeric heavy and light chains, respectively) "are then double transformed into E. coli W3110, the cells grown and the chains reconstituted as set forth in paragraph E.1-E.3 supra." (Page 51, lines 10-12.) In view of the disclosure provided in examples E.1-E.3 and in the rest of example E.4, a person skilled in the art would have had no difficulty at the priority date of the Cabilly application to perform these steps. Figures 2 and 3 of the Cabilly application show the nucleotide and deduced amino acid sequences of mouse anti-CEA antibody κ (light) chain. The nucleotide and amino acid sequences of human κ constant regions were well known in the art at the priority date of the Cabilly application. A person of ordinary skill in the art could have constructed a chimeric light chain expression vector on the analogy of construction of the chimeric heavy chain expression vector. A skilled person could also have performed the subsequent steps of transformation, cultivation, isolation, and reconstitution on the analogy of the detailed description

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provided for the preparation of non-chimeric recombinant antibodies. None of this steps would have required any significant experimentation at the priority date of the Cabilly application.

9. The experimental section describing the preparation of non-chimeric anti-CEA antibody includes coexpression of the antibody heavy and light chains in the same *E. coli* host cell (page 19). The data set forth in the table at page 19 and the Western blot shown in Figure 8C confirm that double transformants have indeed been produced. According to page 22, lines 3-4, the two plasmids containing the coding sequences of chimeric anti-CEA antibody heavy and light chains are "double transformed into E. coli W3110, the cells grown and the chains reconstituted as set forth in paragraph E.1-E.3 supra." A person working in this field could have performed the cotransfection and reconstituted the chimeric chains, following the procedure which has been shown to be successful for non-chimeric antibodies. Opponent VI cited no scientific support for the proposition that the introduction of the chimeric heavy and light chain plasmids into a single bacterial host cell would have resulted in the ejection of one of the chains due to plasmid incompatibility. Plasmid incompatibility plays a role primarily in the absence of selection pressure. From the Cabilly patent it is clear that the double transformants (cotransformed with immunoglobulin heavy and light chains, respectively) when maintained under selection pressure, using different (ampicillin and tetracycline) selection markers resulted in the data shown in the Table on page 19.

10. Following the procedure described in the examples, the recombinant anti-CEA antibodies, including the chimeric antibodies, are obtained in the form of insoluble refractile bodies, and need to be recovered and assembled by cell lysis, solubilization, and subsequent reconstitution. All these steps are described in the Cabilly patent in a detailed manner. Accordingly, a person having the usual skills in molecular biology laboratory techniques in 1983, could have followed the instructions provided in the patent and obtain a chimeric antibody with antigen-specificity by reconstitution of the chimeric antibody chains expressed in the same host cell or in separate host cells. However, based upon

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the totality of the disclosure I conclude that the inventors had also recognized the possibility of secreting the produced antibodies into the periplasmic space of *E. coli*, or into the culture medium, if the host is yeast or a gram positive bacterium (page 11, lines 3-17). Should I have read this passage in 1983, it would have been clear to me that the way to achieve secretion is to replace the native signal sequence of immunoglobulin chains by an *E. coli* signal sequence. A variety of *E. coli* signal sequences were known by early 1983, as attested by the attached review paper: Watson, Nucleic Acids Res. 12:5145 (1984) (Exhibit "H"). Although the latter paper was published after the priority date of the Cabilly patent, most references cited as describing various *E. coli* signals were published prior to or in 1983. For example, the amino acid sequence of the signal peptide of ompA protein was published in 1980 (Movva *et al.*, J. Biol. Chem. 256:27 (1980) (Exhibit "I"). While the selection of the most efficient signal sequence would have involved some experimentation, secretion of at least some chimeric anti-CEA antibody, or antibody fragment (e.g. Fab protein) would have been expected. In addition, at page 8, lines 25-26, the Cabilly patent lists a number of further prokaryotic hosts suitable for antibody production. Of these, the gram positive genus *Bacillus* was shown to secrete large amounts of certain proteins into the medium shortly after the priority date of the Cabilly patent (Palva *et al.*, Gene 22:229 (1983) - Exhibit "J"). *Pseudomonas aeruginosa*, a gram negative bacterium, was also shown to secrete various proteins into the medium (Liu, J. Infect. Dis. 130(Suppl):594 (1974)- Exhibit "K"). These prokaryotes, which are specifically disclosed in the Cabilly patent, would have been further candidates for secretion expression of antibodies or antibody chains or chain combinations.

11. I have reviewed the Declaration of Marc J. Shulman, dated May 21, 1994 along with Exhibits A to E (Shulman I Declaration). I have also reviewed a second Declaration signed by Dr. Shulman on May 15, 1996, along with its Exhibits, including a draft grant application by Gabrielle Boulianne bearing the handwritten date "FEB. '83" and the research report submitted by Dr. Shulman to the Arthritis Society with a letter dated September 30, 1983.

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I did not attend the Ninth Annual Meeting of the Clinical Ligand Assay Society held between March 13 and 17, 1983 in Philadelphia, Pennsylvania, nor have I had the opportunity to speak to anyone who did. I have reviewed the copies of five slides that were reported to have been shown by Dr. Shulman at a lecture delivered at that meeting. The slide entitled "Construction and expression of an Ig Heavy Chain Gene" is a summary of work done by numerous workers in the field of molecular immunology from 1979-1982, including my own work on the structure of the human IgM gene. Indeed, I noticed that Dr. Boulianne refers to my group as the source of human C_κ and C_μ regions shown in the first slide (References 21-23 cited at page 4 of the Boulianne draft). The organization and rearrangement of the genetic elements is summarized in this slide and it contains no novel information, or any detail, statement or suggestion that would lead one to a method of constructing a chimeric antibody. Similarly, the slide entitled "μ Heavy Chain Gene" is a graphic illustration of the genetic organization of the rearranged gene that is found in B cells expressing the IgM antibody. Information summarized in this slide was well known by 1983 and provides no suggestion to prepare chimeric antibodies. The third slide in this series entitled "Expression System" is a schematic of the method published in late 1982 and early 1983 by two independent groups investigating the mechanisms of immunoglobulin gene expression. (Rice and Baltimore, Proc. Natl. Acad. Sci. USA 79:7862 (1982) - M9; Oi *et al.*, Proc. Natl. Acad. Sci. USA 80:825 (1983) - M10). The introduction of heavy or light chain genes into lymphoid cells, derived from myeloma, hybridoma or virally transformed B cell lines results in the expression of these transfected DNA molecules and their assembly with the endogenous heavy or light chain protein into an assembled immunoglobulin. No new information is provided in this figure, and there is nothing present that would be related to the construction and expression of chimeric antibodies. The final two slides in this series relate to "Engineering the V region (by) *In Vitro* Mutagenesis" and "Engineering the C region." These slides reiterate the methods of site-directed mutagenesis and restriction enzyme based methods for reconstructing genes, which were well known in 1983. While the final slide suggests the substitution of other C regions, there is no explanation of what kind of "other regions" would be substituted. Nothing in this slide indicates that the "other C region" could be a human constant region

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substituted for the constant region of a mouse antibody. For example, "engineering the C region" might have meant the generation of immunoglobulin molecules in which the variable regions are identical but the native constant regions are replaced by constant regions of other isotypes, as it was later done, and published by Dangl *et al.* (The EMBO Journal 7:1989 (1988) - Exhibit "L"). There is no novel method being suggested by these slides. No suggestion as to the vector that is to be used is provided, nor is there sufficient detail to determine what Dr. Shulman was proposing to construct and how he was planning to achieve his (unidentified) goal. Accordingly, based upon the slides a person skilled in the art of antibody production in 1983 would not have had a clear understanding of what Dr. Shulman was proposing, even less of how the proposed construct was to be made.

The draft research proposal does not appear to be a public document. It is nonetheless useful in getting a better idea of the approach the laboratory of Dr. Shulman might have been contemplating to construct chimeric immunoglobulin genes, although it is unclear whether any of this has been communicated to the public before the priority date of the Cabilly patent. In the proposal, restriction enzymes were proposed to cut the rearranged heavy and light chain genes in the intron separating the assembled V region and the constant region and then recombined with constant regions derived from cloned human genes. The reassembled genes are inserted into the pSV2 neo vector, which will permit the stable transformation of a hybridoma cell line. Expression of the introduced chimeric gene will depend on the presence of a promoter element on the transferred DNA. The publication which describes the outcome of those experiments, in 1984 (Boulianne *et al.*, Nature 312:643 (1984) - M42), reveals that Dr. Shulman restricted his approach to the expression of chimeric antibodies in lymphoid cells, since those cells retained the transcriptional, translational and assembly machinery to permit the expression of immunoglobulin genes. Similar studies, published in 1984 by Morrison *et al.* (Proc. Natl. Acad. Sci. USA 81:6851 (1984) - M41) use a very similar approach and indicate some of the difficulties inherent with this method.

Expression of immunoglobulin genes in lymphoid cells was found, in 1983, to depend not only on the presence of a promoter upstream of the rearranged V region, but required the presence of an

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"intronic enhancer" residing in the intervening sequence between the assembled V region and the constant region. The presence of this intronic enhancer is required for expression of the transfected immunoglobulin gene. There is no indication in the slides allegedly shown by Dr. Shulman or the draft grant application that inclusion of this element was considered in the construction of their chimeric genes. Furthermore, as was explicitly discussed in the Morrison study, the use of cross-species enhancers resulted in very inefficient expression of the transfected gene, suggesting that for the method to work, the enhancer would have to be of the same species as the recipient lymphoid cell line. Accordingly, even if the structure of an antibody having a human constant region fused to a murine variable region had been clearly communicated in the slides, based upon the information shown, a skilled person would not have been able to make the constructs with a reasonable expectation of success. This is particularly so since the presence of an intronic enhancer is critical to the success of this approach and no mention is made of this element. Therefore, I conclude that the slides of Dr. Shulman, even if accompanied by a limited explanation of the structures shown and the methods contemplated, did not provide sufficient information for the construction of a chimeric gene which would be able to express in a lymphoid cell.

Finally, expression in *E. coli*, yeast or other non-lymphoid eukaryotic cells (which do not ordinarily express an immunoglobulin) was specifically excluded by the approach proposed by Shulman, since the immunoglobulin promoter/enhancer is specific for lymphoid cells and would not function elsewhere. In this regard, I note that the phrase host cell, "which does not ordinarily express an immunoglobulin" (present in Patentee's second subsidiary claim request) clearly means to me non-lymphoid cells which consequently do not have the native machinery to produce immunoglobulins. A lymphoid cell line which is deficient in antibody production would nonetheless be viewed as one which ordinarily expresses immunoglobulin. The interpretation which has been placed on this phrase by Celltech (letter February 20, 1997, section 3-4-3) is not one which would have been adopted by one working in this field. It is clear to me from reading the Cabilly patent (for example from the discussion on page 4, lines 2-25 of the disadvantages of using hybridoma or B cell lines) that part of the point of

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the Cabilly invention was to provide ways for using cells which do not ordinarily produce immunoglobulins and are not "lymphoid" or "myeloid" or "immunocompetent."

In conclusion, I am of the opinion based on the lecture slides, grant application and subsequent publications of Dr. Shulman (without suggesting in any way that any of this information was publicly available at the priority date of the Cabilly patent) that the limited approach he seems to have contemplated was an extension of the studies published in 1982/1983 utilizing transfection of immunoglobulin genes which had been modified by the substitution of the constant region from a human immunoglobulin gene. This approach was limited to a chimeric gene produced in lymphoid cells, and the actual work, published in the December 1984 Boulianne *et al.* paper, was performed in Sp2/0 lymphoid cells, which have the native machinery to produce antibodies, although are actually deficient in the production of both antibody heavy and light chains. None of the documents studied have any suggestion that the production of chimeric antibodies in cells other than lymphoid cells was contemplated or would have been expected to be possible given the specific specialization of lymphoid cells for the expression and assembly of antibody molecules.

12. I have read the grant application submitted by Dr. Leonard Herzenberg on 2/28/82 (Application No: 2 RO1 CA04681-24) entitled "Genetic Studies with Mammalian Cells." In this application, Dr. Herzenberg summarizes the past progress of his research group on the analysis of lymphoid cells and lymphoid cell proteins using the technique he pioneered, "fluorescence activated cell sorting" or FACS. Dr. Herzenberg proposes experiments for the coming granting period that would extend on his ongoing studies. In particular in his "Research Plan" he mentioned under specific aim 1 a goal of selecting interchromosomal (cross allotype) and interspecies (mouse-human) switch variants producing chimeric antibodies. On pages 34-37 of this application the rationale and approach Dr. Herzenberg is considering is described in somewhat greater detail, although still remains rather sketchy. The experimental question which is being considered in this goal is the molecular explanation of the appearance of immunoglobulin switch variants and, in particular, switches which appear to defy the

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then-known genetic structure of the heavy chain locus. Switching refers to the process wherein one constant region is naturally substituted with another constant region while retaining the same assembled variable region. This phenomenon occurs during the maturation of an immune response, where antibodies of the IgM class are produced first, followed by IgG antibodies, with the same specificity for antigen. This switching (isotype or class switching) was believed to proceed in an orderly fashion, following the order of constant region genes on the chromosome, i.e. μ, δ, $\gamma 3$, $\gamma 1$, $\gamma 2\beta$, $\gamma 2\alpha$, ε, α. Switching from μ to $\gamma 1$ results in the deletion of the $\gamma 3$ gene. Dr. Herzenberg identified a switch variant that appeared to go backwards, that is from expressing $\gamma 2\beta$ to $\gamma 1$. He wished to understand how such a switch was possible and proposed testing the hypothesis that this switch involved interchromosomal rearrangement, in contrast to the usual intrachromosomal rearrangement. As he states "our major impetus, however, derived from the potential we saw in such variants for answering a variety of questions about antibody molecules and the genetic mechanisms that control their production."

To address this question, Dr. Herzenberg proposed to select for naturally occurring switch variants using hybridoma fusions between lymphoid cells which would allow him to distinguish between inter and intra chromosomal events. Two approaches are considered. In the first, the allotypic differences (i.e. the two chromosomes bear different alleles of the same gene) which can be distinguished by FACS analysis. In the second approach, the two chromosomes are derived from different species, i.e. mouse and human. Hybridoma type fusions between mouse and human lymphoid cells were proposed as the starting point for searching for cells which may have undergone interchromosomal exchange during isotype switching and which would therefore be predicted to appear as mouse-human chimeras.

To the best of my knowledge, judged from subsequent studies published by Dr. Herzenberg (including documents M35, M39, M41, Oi *et al.*, "Hybridoma Antibody-Producing Switch Variants: A Variant Lacking the CH1 Domain, pp. 281-287, In: Cell Fusion: Gene Transfer and Transformation, Beers and Basset, eds., Raven Press, New York, 1984 (Exhibit "M"), and Herzenberg *et al.*, J. Cell. Biochem. 13th Annual UCLA Symposia, Abstracts, Supplement 8A, 1984, Abstract # 0443 (Exhibit

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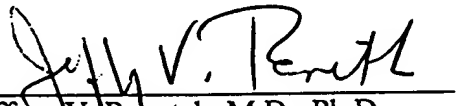
"N"), it appears that cross-species switch variants have not been detected. Indeed, in view of their later filed patent application (EP 173494A - P3), instead of finding naturally occurring switch variants, in an attempt to make chimeric antibodies Dr. Herzenberg ultimately relied on an approach similar to that published by Dr. Shulman and others, in which a gene was constructed with mouse V region and human C region and transfected into lymphoid cells to obtain expressed antibodies. The grant proposal does not address this method at all. Rather it concentrates on a hypothetical genetic pathway and means for detecting events that would support its existence. It does not instruct one skilled in the art in constructing and expressing chimeric antibodies.

13. In reaching the opinions set forth in this Declaration, I rely upon my education, my research experiences in immunology and biochemistry and my familiarity with the academic and patent literature in my field both today and as existed at the priority date of the Cabilly patent.

14. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.



Jeffrey V. Ravetch, M.D., Ph.D.

Date: 3/14/97